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## Mortality prediction using modern peptide biomarkers in hemodialysis patients: a comparative analysis

Artunc, F ; Nowak, Albina ; Müller, Christian ; Peter, Andreas ; Heyne, Nils ; Häring, Hans-Ulrich ; Friedrich, Björn

**Abstract:** BACKGROUND/AIMS: Determination of peptide biomarkers such as troponins, natriuretic peptides or the recently reported FGF23 can be useful to identify hemodialysis patients with a high risk of mortality. However, it is desirable to focus on few robust parameters to warrant their routine application. METHODS: In a prospective cohort study with 239 prevalent hemodialysis patients we studied the prognostic significance of 10 simultaneously determined modern peptide biomarkers (high sensitive troponin I and T, NT-pro-BNP, BNP, MR-pro-ANP, MR-pro-ADM, CT-pro-ET1, copeptin, FGF23 and a-Klotho) and compared them with parameters traditionally associated with mortality (PTH, Ca, Pi, albumin, CRP, cholesterol, AP). RESULTS: After a follow-up of 4 years, plasma concentration of troponins, natriuretic peptides, MR-pro-ADM, FGF23 as well as PTH, CRP, AP were significantly higher in deceased patients (n=95). Hazard ratios from cox regression on a continuous scale (doubling of plasma concentration) or relative in tertiles were highest for high sensitive troponins, followed by natriuretic peptides and MR-pro-ADM (1.6-2.0 and 2.3-5.5, resp.). C-indices were also highest for troponins (0.708-0.746), followed by natriuretic peptides (0.706-0.731). Traditional parameters had low c-indices (0.598-0.655). Stepwise cox regression revealed that among all parameters troponin I, NT-pro-BNP, PTH and CRP remained independent predictors of mortality and a composite score had the highest c-index (0.799 [0.740-0.849]). CONCLUSIONS: Among peptide biomarkers high sensitive troponins and to a lesser extent natriuretic peptides are strong predictors of mortality in asymptomatic hemodialysis patients, followed by markers of mineral-bone disease and inflammation.

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Original Paper

# Mortality Prediction Using Modern Peptide Biomarkers in Hemodialysis Patients – A Comparative Analysis

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## Key Words

Biomarkers • Hemodialysis • Mortality • Peptide • Troponin

## Abstract

**Background/Aims:** Determination of peptide biomarkers such as troponins, natriuretic peptides or the recently reported FGF23 can be useful to identify hemodialysis patients with a high risk of mortality. However, it is desirable to focus on few robust parameters to warrant their routine application. **Methods:** In a prospective cohort study with 239 prevalent hemodialysis patients we studied the prognostic significance of 10 simultaneously determined modern peptide biomarkers (high sensitive troponin I and T, NT-pro-BNP, BNP, MR-pro-ANP, MR-pro-ADM, CT-pro-ET1, copeptin, FGF23 and a-Klotho) and compared them with parameters traditionally associated with mortality (PTH, Ca, Pi, albumin, CRP, cholesterol, AP). **Results:** After a follow-up of 4 years, plasma concentration of troponins, natriuretic peptides, MR-pro-ADM, FGF23 as well as PTH, CRP, AP were significantly higher in deceased patients (n=95). Hazard ratios from cox regression on a continuous scale (doubling of plasma concentration) or relative in tertiles were highest for high sensitive troponins, followed by natriuretic peptides and MR-pro-ADM (1.6-2.0 and 2.3-5.5, resp.). C-indices were also highest for troponins (0.708-0.746), followed by natriuretic peptides (0.706-0.731). Traditional parameters had low c-indices (0.598-0.655). Stepwise cox regression revealed that among all parameters troponin I, NT-pro-BNP, PTH and CRP remained independent predictors of mortality and a composite score had the highest c-index (0.799 [0.740-0.849]). **Conclusions:** Among peptide biomarkers high sensitive troponins and to a lesser extent natriuretic peptides are strong predictors of mortality in asymptomatic hemodialysis patients, followed by markers of mineral-bone disease and inflammation.

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## Introduction

Accurate risk prediction and identification of asymptomatic hemodialysis patients with a high risk of mortality is an important clinical task. In addition to many clinical or functional variables associated with mortality such as age [1], dialysis vintage [2], cardiac function [3] and physical status /frailty [4], laboratory parameters have also been shown to predict mortality. 1990 Lowrie et al. first published that albumin, creatinine, cholesterol and calcium among others were predictors of mortality in hemodialysis patients [5]. Since then, numerous other laboratory parameters have been found to be associated with mortality including c-reactive protein (CRP) [6, 7], interleukin-6 [8], parathyroid hormone (PTH) [9], homocysteine [10], troponins [11], natriuretic peptides [12, 13] and most recently fibroblast growth factor 23 (FGF23) [14]. Many of these parameters are peptides that are secreted in increased amounts during the course of end-stage renal disease: they can be termed as biomarkers because their plasma concentration is linked to severity of disease state and outcome [15]. In particular, cardiac biomarkers such as troponins and natriuretic peptides are among the strongest biomarkers in maintenance hemodialysis patients. In their meta-analyses, Khan et al. and Cheng et al. found that an elevated plasma troponin and natriuretic peptide concentrations were associated with a 2.64- and 3.85-fold increase, respectively, in the relative risk for all-cause mortality, [16, 17].

Despite their prognostic significance, many peptide biomarkers are not utilized in daily practice in contrast to common parameters such as parathyroid hormone, albumin or creatinine for several reasons. First, many peptide biomarkers are not required to guide renal replacement therapy and the treatment of other common complications of ESRD patients including renal anemia or mineral-bone disease. Second, the interpretation of their plasma concentrations is complex because hemodialysis patients often exhibit high plasma concentrations making it difficult to distinguish between mere accumulation and increased release. Third, it is unclear which biomarker the clinician should rely on as there are many candidates which differ in their strength of association with mortality and studies describing the prognostic significance of biomarkers with a c-statistic or hazard ratios cannot be compared directly. Thus, from a clinician's point of view it would be highly desirable to focus on single parameters with a high predictive value that are validated in hemodialysis patients with specific cut-off values.

In previous studies, our group has investigated the performance of various modern peptide biomarkers in stable hemodialysis patients that were newly developed and had been scarcely tested in this cohort. These studies included high sensitive troponins T and I, natriuretic peptides (MR-pro-ANP, BNP, NT-pro-BNP), vasoactive peptides (coceptin, MR-pro-adrenomedullin, CT-pro-endothelin), FGF23 and alpha-Klotho [18-21]. With the current study, we wanted to find out, which of these biomarkers could qualify for risk prediction on a routine basis in asymptomatic hemodialysis patients. To this end we compared the performance of ten of these modern peptide biomarkers that were measured in the same hemodialysis cohort at the same time point with regard to the prediction of mortality during a 4.4-year follow-up and compared them to traditional laboratory markers associated with mortality.

## Subjects and Methods

### *Patients and cohort*

This prospective multicenter study included stable ambulatory hemodialysis patients from four dialysis centers in Southwest Germany between September 2009 and April 2010. Patients were included after they provided written informed consent and if there was no evidence of an acute illness, a cardiac event or a procedure within the previous two months. Patients with cardiac diseases leading to increased cardiac biomarkers concentration (troponin, natriuretic peptides) independent of ESRD, such as amyloidosis

were excluded. The study was approved by the local ethics committee of the University of Tuebingen (191/2009B02).

#### *Laboratory assays*

Plasma concentrations of the parameters were measured in three independent samples that were taken within two weeks prior to the start of a dialysis session. Blood was collected in lithium-heparinized tubes (Sarstedt, Nuembrecht, Germany), cooled at 4°C and centrifuged within four hours; plasma samples were stored at -80°C for further analysis. The plasma concentrations of troponin I and T were measured using the troponin I Ultra assay on a Siemens ADVIA Centaur system (Siemens Healthcare Diagnostics, Eschborn, Germany) and using the high sensitive troponin T assay on an Elecsys 2010 system (Roche, Basel, Switzerland), respectively. BNP and NT-pro-BNP were measured on a Siemens ADVIA Centaur and Siemens Immulite 2000 Xpi system, respectively, as specified by the manufacturers. MR-pro-ANP, copeptin, MR-pro-ADM and CT-pro-ET1 were measured using an automated immunoluminometric assay on a Kryptor system (B.R.A.H.M.S AG, Henningsdorf, Germany) as previously described in [22-24]. Plasma FGF23 and alpha-Klotho concentration were manually measured using ELISA methods (Immuno-Biologic Laboratories Co., Ltd. Japan; and Immutopics, San Clemente, CA, USA, respectively) [21]. Plasma beta-2-microglobulin concentrations were measured using a turbidimetric assay (Randox Laboratories, Antrim, United Kingdom). All other laboratory values (i.e., parathyroid hormone, hemoglobin, albumin, C-reactive protein, calcium, alkaline phosphatase, total cholesterol and inorganic phosphorus) were extracted from the patients' medical records and averaged from the available values of the previous year (4 – 12 values).

#### *Statistical analysis*

Three plasma samples were available from 87% of the patients and were averaged to calculate the arithmetic mean without excluding possible outliers. Plasma concentrations were log10 transformed to approximate normal distribution. The follow-up period started on the first day of blood draw and lasted until August 25<sup>th</sup>, 2014. Patients receiving renal transplantation were censored at the day of transplantation. Causes of death were classified according to the best knowledge of each particular case. To analyze cardiovascular mortality separately, the follow-up of the patients who died from non-cardiovascular death was censored at the day of their death taking into account that patients dying from non-cardiovascular causes are no longer at risk of dying from cardiovascular causes. The averaged values of the deceased patients were compared to those from the surviving patients using a two-sided t-Test. Mortality was analyzed in quintiles to rule out a j-curved relationship which would hinder Cox proportional regression analysis. Univariate and multivariate proportional hazards were then calculated using the Cox proportional regression analysis. Prognostic performance was analyzed using the c-index. Cut-off values were calculated by maximizing sensitivity and 1-specificity (Youden index). C-indices were compared using the method of Hanley & McNeil [25]. Calibration of the models was tested using the Hosmer-Lemeshow-Test.

Data analysis was performed using the statistical software package MedCalc Statistical Software version 13.3 (MedCalc Software bvba, Ostend, Belgium) and JMP 10.0.1 (SAS Institute, Cary, NC, USA). A p-value <0.05 was considered statistically significant.

## **Results**

### *Patients*

From a total of n = 250 patients treated in the participating centers, 239 patients (95.6%) were included in the study. 6 patients declined written informed consent, 2 died within the period of blood sampling, 2 had cardiac amyloidosis and one patient was only recently started on dialysis. The baseline characteristics of the study cohort are given in table 1.

### *Association with mortality*

After 4.4 years, follow-up data on all 239 patients were available (median follow-up time 1676 days [IQR 1623; 1727]). Of these patients, 95 died (40%) and 17 (7%) received kidney transplantation (table 2). A comparison of the median plasma concentrations of the

studied laboratory parameters between survivors and deceased patients is shown in table 3. Among modern biomarkers, all parameters related to cardiac health were highly different as well as MR-pro-ADM and FGF23, a parameter related to mineral bone. Among the traditional parameters CRP, PTH, AP, but not albumin, were higher in the deceased patients than in survivors. All of parameters had a linearly increasing relationship with mortality.

## Prognostic value of single parameters

To compare the strength of the relationship of the individual parameter with all-cause mortality, hazard ratios (HR) from cox regression analysis were calculated on both a continuous scale and a relative scale based on tertiles. Although HR values on a continuous scale are difficult to directly compare due to differences in the variability and amplitude of each parameter, HR values in relation to the lowest tertile of each marker might circumvent this. As shown in Fig 1, unadjusted HR for the doubling of the plasma concentration were highest for troponin T, followed by MR-pro-ADM, MR-pro-ANP and troponin I. When adjusting

**Table 1.** Baseline characteristics of the cohort (N=239). Values are shown as median and interquartile range for continuous variables and as percentages for categorical variables.

Age	70 (61; 77) years
gender distribution	64 % male (n= 153) / 36 % female (n= 86)
Cause of renal disease	
diabetic nephropathy	26 % (n= 63)
arterial hypertension	8 % (n= 19)
glomerulonephritis	30 % (n= 71)
polycystic kidney disease	5 % (n= 11)
other / unknown	31 % (n= 75)
dialysis vintage	46 (19; 85) months
duration of dialysis session	4.0 (4.0; 4.5) hours
dialysis access	
arteriovenous fistula	71 % (n= 169)
PTFE graft	13 % (n= 31)
tunnelled catheter	16 % (n= 38)
dialysis membrane	
high-flux	92 % (n= 219)
low-flux	8 % (n= 20)
residual diuresis	250 (0; 1000) mL / day
blood pressure	134 (122; 144) / 69 (63; 74) mm Hg
singe pool Kt / V	1.55 (1.40; 1.73)
medication	
phosphate binders	86% (n=205)
vitamin D replacement	97% (n=233)
ACE-I or ARB	53% (n=126)
β-Blockers	64% (n=153)
Aspirin	42% (n=101)
anticoagulant or clopidogrel	28 % (n=67)
statin	42% (n=101)

**Table 2.** Follow-up data after 4.6 (IQR 4.4;4.7) years and causes of death

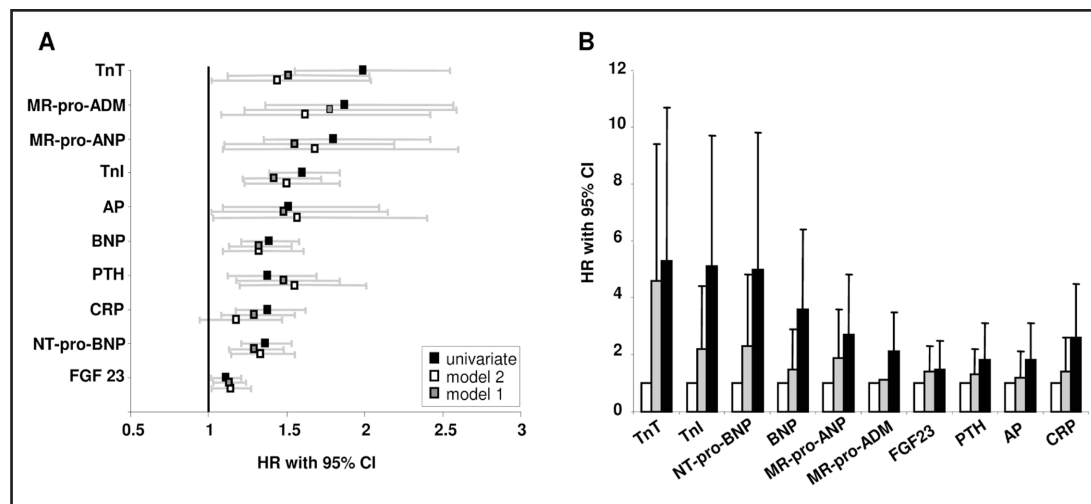
continued on HD	127 (53% of the cohort)
transplanted	17 (7%)
deceased	95 (40%)
sudden death	18 (19% of all deaths)
cardiovascular death§	19 (20%)
infection and sepsis	14 (15%)
malignancy	6 (6%)
wasting / cachexia	26 (27%)
others / unknown	12 (13%)

§ composite of deaths due to coronary artery disease, hypertension, peripheral artery disease.



**Table 3.** Plasma concentrations of laboratory parameters in the whole cohort and in survivors versus deceased patients. Values are shown as median with interquartile range.

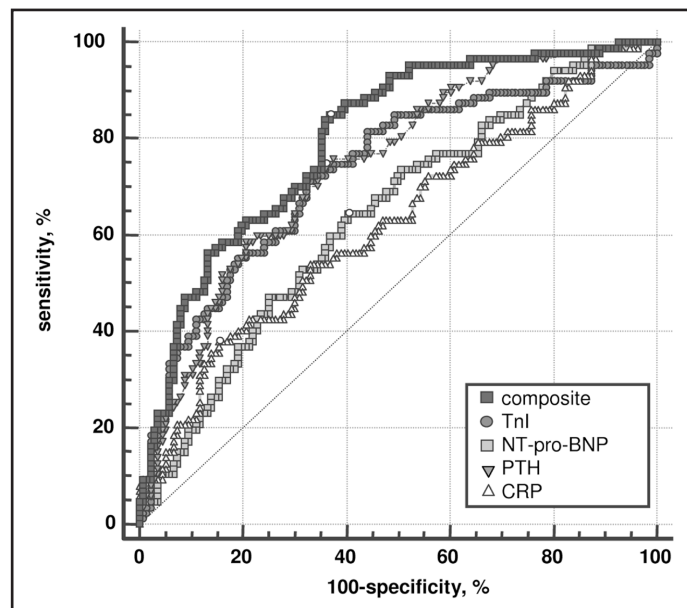
	all (n=239)	survivors (n=145)	deceased (n=95)	p-Value
modern biomarkers				
troponin T, pg/ml	49 (31; 73)	39 (27; 67)	64 (44; 84)	<0.0001
troponin I, pg/ml	14 (7; 28)	11 (6; 19)	24 (13; 39)	<0.0001
NT-pro-BNP, pg/ml	4435 (1687; 16228)	2697 (1282; 7207)	11642 (3826; 26555)	<0.0001
BNP, pg/ml	337 (146; 684)	236 (104; 470)	565 (266; 1515)	<0.0001
MR-pro-ANP, pM	907 (650; 1298)	788 (589; 1160)	1112 (786; 1460)	<0.0001
MR-pro-ADM, pM	1.26 (1.02; 1.80)	1.27 (0.99; 1.66)	1.50 (1.08; 2.25)	0.0004
copeptin, pM	132 (78; 192)	127 (81; 183)	147 (73; 198)	0.1677
CT-pro-ET1, pM	149 (121; 181)	148 (121; 182)	150 (117; 180)	0.6763
FGF23, RU/ml	240 (95; 737)	208 (84; 714)	289 (121; 1029)	0.0194
$\alpha$ -Klotho, pg/ml	339 (261; 426)	339 (259; 421)	349 (260; 431)	0.7476
traditional parameters				
PTH, pg/ml	204 (130; 348)	187 (120; 300)	252 (149; 384)	0.0004
Ca, mM	2.30 (2.20; 2.40)	2.30 (2.22; 2.40)	2.28 (2.18; 2.38)	0.9699
Pi, mM	1.60 (1.38; 1.88)	1.56 (1.39; 1.86)	1.62 (1.37; 1.88)	0.6103
AP, U/l	81 (63; 108)	76 (60; 101)	88 (67; 115)	0.0096
CRP, mg/l	8.6 (4.6; 15.0)	7.2 (3.9; 11.7)	11.4 (6.9; 19.2)	<0.0001
Hb, g/dl	11.5 (11.1; 12.0)	11.5 (11.1; 12.0)	11.4 (11.1; 12.0)	0.2975
albumin, g/l	37 (35; 39)	37 (36; 40)	37 (35; 39)	0.8266
cholesterol, mg/dl	167 (143; 196)	167 (143; 196)	165 (139; 196)	0.6206
$\beta$ 2-microglobulin, mg/l	24 (19; 25)	23 (20; 25)	24 (19; 25)	0.7340



**Fig. 1.** Hazard ratios (and 95% CIs) for all-cause mortality on a continuous scale (A) for a two-fold increase and on a relative scale with the lowest tertile serving as reference (B). Model 1 is adjusted for demographics, blood pressure and dialysis-specific risk factors (time on dialysis, residual diuresis, vascular access and single pool Kt/V). Model 2 is adjusted for all factors associated with mortality (significant difference between deceased and surviving patients): age, body mass index, duration of HD session, diastolic blood pressure, comorbidities: systolic dysfunction (1=yes), peripheral vascular disease (1=yes), coronary artery disease (1=yes), atrial fibrillation (1=yes).

for different demographic (model 1) or survival-associated variables (model 2), HR values were attenuated, but still were significantly different from unity in almost all studied parameters. When analyzing unadjusted and adjusted HR values normalized to the lowest tertile of each parameter, troponin T and I again had the highest values, followed by the natriuretic

**Fig. 2.** ROC curves showing the prognostic performance of predicting all-cause mortality for troponin I, NT-pro-BNP, CRP and PTH as well as a composite score of these four parameters. Note that the c-index of the composite model (0.799) is significantly higher than that derived from each parameter (TnI 0.746; NT-pro-BNP 0.731, CRP 0.655, PTH 0.614).



peptides indicating a strong relationship of all-cause mortality with these parameters. C-indices of the parameters associated with mortality were calculated to analyze the prognostic performance regarding the discrimination of deceased patients. Troponins showed the highest values for the c-index, followed by natriuretic peptides. Among traditional biomarkers, CRP had the highest c-index. When analyzing cardiovascular mortality, c-indices were slightly higher, particularly for the cardiac biomarkers. Interestingly, c-index of CRP was lower indicating an attenuated relationship to cardiovascular mortality (table 4).

Calibration of the parameters with regard to the prediction of risk compared the observed risks was tested using the Hosmer-Lemeshow test. Both modern biomarkers as well as traditional parameters showed a good calibration (p values from 0.10-0.98 and 0.57-0.88, respectively).

#### *Prognostic value of multiple parameters*

To find out parameters independently associated with mortality that could be summarized into a composite risk score, a proportional cox regression with a stepwise approach and retention if  $p < 0.05$  was performed. Among all of the parameters listed in table 3, four parameters were highly independent predictors of mortality and could be combined in a multivariate model: troponin I ( $p < 0.0001$ ), NT-pro-BNP ( $p = 0.0290$ ), CRP ( $p = 0.0072$ ) and parathyroid hormone ( $p = 0.0064$ ). Using a calculated score derived from troponin I (beta 1.1293), NT-pro-BNP (beta 0.5098), CRP (beta 0.8251) and parathyroid hormone (beta 0.9812), the c-index could be improved up to 0.799 [0.740-0.849], a value statistically higher than the respective value derived from single parameters (p between  $< 0.0001$  and 0.0131; Fig 2). The cut-off value was 6.0498 and had a sensitivity and specificity of 85% and 63%, respectively. The calculated score correctly reclassified 3%, 3%, 10% and 6%, of the patients compared to using trop I, NT-pro-BNP, CRP and PTH alone.

When analyzing cardiovascular mortality as endpoint in a stepwise multivariate Cox regression model, only troponin I ( $p = 0.0008$ ), BNP ( $p = 0.0095$ ) and parathyroid hormone ( $p = 0.0370$ ) were independent predictors. Using a calculated score derived from these, the c-index could be improved up to 0.804 [0.748-0.852]. The cut-off value was 8.5523 and had a sensitivity and specificity of 68% and 85%, respectively.



**Table 4.** C-statistics of the parameters associated with all-cause and cardiovascular (CV) mortality.

Parameter	AUC for all-cause mortality with 95% CI	cutoff	sensitivity %	specificity %	PPV %	NPV %	AUC for CV-mortality with 95% CI
modern biomarkers							
troponin T	0.708 (0.646-0.765)	>37 pg/ml	88	49	53	86	0.741 (0.681-0.796)
troponin I	0.746 (0.686-0.800)	>14 pg/ml	74	65	58	79	0.769 (0.711-0.821)
NT-pro-BNP	0.731 (0.668-0.788)	>4916 pg/ml	70	68	59	77	0.745 (0.683-0.801)
BNP	0.706 (0.644-0.763)	>529 pg/ml	54	82	66	73	0.754 (0.695-0.807)
MR-pro-ANP	0.666 (0.602-0.725)	>983 pM	62	67	55	73	0.727 (0.665-0.782)
MR-pro-ADM	0.635 (0.571-0.696)	>1.63 pM	47	74	54	68	0.664 (0.600-0.723)
FGF23	0.572 (0.507-0.636)	>125 RU/ml	56	58	47	67	0.597 (0.532-0.660)
traditional parameters							
PTH	0.614 (0.549-0.676)	>351 pg/ml	37	84	60	67	0.618 (0.553-0.680)
AP	0.598 (0.533-0.661)	>80 U/l	61	56	48	69	0.604 (0.538-0.666)
CRP	0.655 (0.591-0.715)	>8.2 mg/l	67	58	51	73	0.618 (0.553-0.680)
PPV positive predictive value, NPV negative predictive value.							

## Discussion

This study allows for the direct quantitative comparison of different peptide biomarkers in predicting mortality in chronic hemodialysis patients. The main finding was that high sensitive cardiac troponins and natriuretic peptides were the strongest predictors of mortality among the tested biomarkers. In the absence of acute myocardial injury or ischemia, cardiac troponins and natriuretic peptides are robust markers of cardiac dysfunction and reflect myocardial integrity in chronic hemodialysis patients. In a previous study with this cohort, systolic left ventricular function estimated from echocardiography was the main determinant of the plasma concentration of cardiac biomarkers and also the strongest independent predictor of mortality [18, 20]. Among cardiac biomarkers, troponin T and NT-pro-BNP revealed largely elevated plasma concentration due to accumulation as excretion was related to residual renal function in contrast to troponin I [18, 19]. Along the same lines, the cut-off of troponin T and NT-pro-BNP derived from c-indices was higher than used for non-HD patients (Table 4). For troponin I, the cut-off was 14 pg/ml, which was still lower than the 99<sup>th</sup> percentile that is used as a threshold for the diagnosis of acute myocardial infarction.

Among the other modern peptide biomarkers, only MR-pro-ADM and FGF23 showed an association with mortality and among the traditional biomarkers, parathyroid hormone, AP and CRP were associated with mortality. However, their association was rather weak and showed great overlap between survivors and deceased patients (table 3) which precludes their use for risk stratification and prognostication. According to Swets [26], a good diagnostic test is characterized by a c-statistic value above 0.8 and only the composite measure consisting of troponin I, NT-pro-BNP, parathyroid hormone and CRP approached this value. Due to their low sensitivity and specificity, biomarkers tested in this study performed better in identifying low risk patients compared to high risk patients (higher negative predictive values).

The findings in this study underscore the utmost importance of cardiac health in the prognosis of hemodialysis patients as cardiac biomarkers reflect a failing heart and features of the renocardiac syndrome. Troponins measured with a high sensitive assay may provide high resolution to identify patients at high risk. Interestingly, prognostication could be significantly improved by adding parathyroid hormone and CRP to the model. This points to mineral bone disorder and chronic inflammation as additional areas highly relevant for the mortality risk of asymptomatic hemodialysis patients. In this study, parathyroid hormone was the only independent predictor among the markers of bone mineral disorder and outperformed FGF23. This interesting finding indicates that secondary hyperparathyroidism is a potent surrogate of CKD-MBD-associated mortality in hemodialysis patients justifying the current practice of routinely measuring PTH. An explanation for this might be the delayed kinetic of parathyroid hormone secretion that relies on more profound changes in the calcium and phosphorus metabolism compared to FGF23 that is more volatile. In our data, this was reflected by a higher coefficient of variation (CV) of FGF compared to PTH (30% vs. 16%).

Another important predictor of mortality, albumin, did not play a role in this cohort although there were patients with low albumin concentration (25% below 35 g/l) that should be at high risk of mortality. In the study of Kalantar-Zadeh [27], baseline albumin concentration was an independent predictor of mortality in hemodialysis patients. However, in the same study, time-varying albumin concentration based on quarterly measurements of serum albumin (up to 8 albumin values per patient over 2 years) substantially lost its strength of association to mortality [27]. In our study, albumin concentrations were derived from the average of the values available from the patient's medical records (4-6 values) which resembled time-varying values.

The limitations of this study may be its relatively small sample size compared to data from randomized controlled trials (RCT) such as the 4D study [28] or large registries such as the DOPPS that were able to disclose even weak prognostic associations [29, 30]. In the above mentioned study by Kalantar-Zadeh et al. [27], the association of albumin concentration and mortality was analyzed in a sample of 58 058 patients yielding a high statistical power to find significant associations even with small HR values. However, no c-indices are usually provided in these large studies that could tell the clinician how predictive the concentration of the tested laboratory marker is for the individual patient. In a new analysis of the DOPPS study, a model with serum albumin and age reached a c-statistic of 0.72 [31]. Thus, associations seen in large studies are often not visible in the routine practice of nephrologists. Additionally, strict inclusion criteria often impair the generalizability of results from RCTs. In this cohort, there were minimal inclusion criteria, and the results thus more likely reflect the daily practice pattern of a nephrologist. Due to the small size of our cohort we could not divide the sample into a training and validation set, hence our findings must be validated in other independent hemodialysis cohorts. Another limitation is that not all analyzed parameters were measured from the same sample, and values for PTH, CRP and albumin were extracted from the records.

Our study shows that the prognostic information that can be obtained from peptide biomarkers is very high for cardiac markers making them a candidate for routine clinical risk stratification although there is no evidence-based strategy how to improve outcome while lowering plasma concentrations. Additionally, there are virtually no data on the prognostic association of the change of cardiac biomarkers with time, and this study also was based on the measurement of the biomarkers at one time point. Thus, studies on the longitudinal course of biomarkers would be helpful to decide about the clinical usefulness of measuring biomarkers on a routine basis.

## Conclusion

Among modern peptide biomarkers, high sensitive troponins and to a lesser extent natriuretic peptides are strong predictors of mortality in asymptomatic hemodialysis patients, followed by markers of mineral-bone disease and inflammation.

## Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

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## References

- 1 Mailloux LU, Bellucci AG, Napolitano B, Mossey T, Wilkes BM, Bluestone PA: Survival estimates for 683 patients starting dialysis from 1970 through 1989: identification of risk factors for survival. *Clin Nephrol* 1994;42:127-135.
- 2 Chertow GM, Johansen KL, Lew N, Lazarus JM, Lowrie EG: Vintage, nutritional status, and survival in hemodialysis patients. *Kidney Int* 2000;57:1176-1181.
- 3 Cheung AK, Sarnak MJ, Yan G, Berkoben M, Heyka R, Kaufman A, Lewis J, Rocco M, Toto R, Windus D, Ornt D, Levey AS; HEMO Study Group: Cardiac diseases in maintenance hemodialysis patients: results of the HEMO Study. *Kidney Int* 2004;65:2380-2389.
- 4 Johansen KL, Kaysen GA, Dalrymple LS, Grimes BA, Glidden DV, Anand S, Chertow GM: Association of physical activity with survival among ambulatory patients on dialysis: the Comprehensive Dialysis Study. *Clin J Am Soc Nephrol* 2013;8:248-253.
- 5 Lowrie EG, Lew NL: Death risk in hemodialysis patients: the predictive value of commonly measured variables and an evaluation of death rate differences between facilities. *Am J Kidney Dis* 1990;15:458-482.
- 6 Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C: Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. *Kidney Int* 1999;55:648-658.
- 7 Honda H, Qureshi AR, Heimbürger O, Barany P, Wang K, Pecoits-Filho R, Stenvinkel P, Lindholm B: Serum albumin, C-reactive protein, interleukin 6, and fetuin A as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. *Am J Kidney Dis* 2006;47:139-148.
- 8 Rao M, Guo D, Perianayagam MC, Tighiouart H, Jaber BL, Pereira BJ, Balakrishnan VS: Plasma interleukin-6 predicts cardiovascular mortality in hemodialysis patients. *Am J Kidney Dis* 2005;45:324-333.
- 9 Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM: Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J Am Soc Nephrol* 2004;15:2208-2218.
- 10 Heinz J, Kropf S, Luley C, Dierkes J: Homocysteine as a risk factor for cardiovascular disease in patients treated by dialysis: a meta-analysis. *Am J Kidney Dis* 2009;54:478-489.
- 11 Apple FS, Murakami MM, Pearce LA, Herzog CA: Predictive value of cardiac troponin I and T for subsequent death in end-stage renal disease. *Circulation* 2002;106:2941-2945.
- 12 Zoccali C, Mallamaci F, Benedetto FA, Tripepi G, Parlongo S, Cataliotti A, Cutrupi S, Giaccone G, Bellanuova I, Cottini E, Malatino LS; Creed Investigators: Cardiac natriuretic peptides are related to left ventricular mass and function and predict mortality in dialysis patients. *J Am Soc Nephrol* 2001;12:1508-1515.

- 13 Madsen LH, Ladefoged S, Corell P, Schou M, Hildebrandt PR, Atar D: N-terminal pro brain natriuretic peptide predicts mortality in patients with end-stage renal disease in hemodialysis. *Kidney Int* 2007;71:548-554.
- 14 Gutierrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, Shah A, Smith K, Lee H, Thadhani R, Jüppner H, Wolf M: Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med* 2008;359:584-592.
- 15 Strimbu K, Tavel JA: What are biomarkers? *Curr Opin HIV AIDS* 2010;5:463-466.
- 16 Khan NA, Hemmelgarn BR, Tonelli M, Thompson CR, Levin A: Prognostic value of troponin T and I among asymptomatic patients with end-stage renal disease: a meta-analysis. *Circulation* 2005;112:3088-3096.
- 17 Cheng YJ, Yao FJ, Liu LJ, Tang K, Lin XX, Li WJ, Zhang J, Wu SH: B-type natriuretic peptide and prognosis of end-stage renal disease: a meta-analysis. *PLoS One* 2013;8:e79302.
- 18 Artunc F, Mueller C, Breidthardt T, Twerenbold R, Peter A, Thamer C, Weyrich P, Haering HU, Friedrich B: Sensitive troponins--which suits better for hemodialysis patients? Associated factors and prediction of mortality. *PLoS One* 2012;7:e47610.
- 19 Artunc F, Mueller C, Breidthardt T, Twerenbold R, Rettig I, Usta E, Häring HU, Friedrich B: Comparison of the diagnostic performance of three natriuretic peptides in hemodialysis patients: which is the appropriate biomarker? *Kidney Blood Press Res* 2012;36:172-181.
- 20 Artunc F, Nowak A, Mueller C, Breidthardt T, Twerenbold R, Wagner R, Peter A, Haering HU, Ebmeyer S, Friedrich B: Plasma concentrations of the vasoactive peptide fragments mid-regional pro-adrenomedullin, C-terminal pro-endothelin 1 and copeptin in hemodialysis patients: associated factors and prediction of mortality. *PLoS One* 2014;9:e86148.
- 21 Nowak A, Friedrich B, Artunc F, Serra AL, Breidthardt T, Twerenbold R, Peter M, Mueller C: Prognostic value and link to atrial fibrillation of soluble Klotho and FGF23 in hemodialysis patients. *PLoS One* 2014;9:e100688.
- 22 Morgenthaler NG, Struck J, Alonso C, Bergmann A: Measurement of midregional proadrenomedullin in plasma with an immunoluminometric assay. *Clin Chem* 2005;51:1823-1829.
- 23 Morgenthaler NG, Struck J, Alonso C, Bergmann A: Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. *Clin Chem* 2006;52:112-119.
- 24 Papassotiriou J, Morgenthaler NG, Struck J, Alonso C, Bergmann A: Immunoluminometric assay for measurement of the C-terminal endothelin-1 precursor fragment in human plasma. *Clin Chem* 2006;52:1144-1151.
- 25 Hanley JA, McNeil BJ: A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology* 1983;148:839-843.
- 26 Swets JA: Measuring the accuracy of diagnostic systems. *Science* 1988;240:1285-1293.
- 27 Kalantar-Zadeh K, Kilpatrick RD, Kuwae N, McAllister CJ, Alcorn H Jr, Kopple JD, Greenland S: Revisiting mortality predictability of serum albumin in the dialysis population: time dependency, longitudinal changes and population-attributable fraction. *Nephrol Dial Transplant* 2005;20:1880-1888.
- 28 Wanner C, Krane V, März W, Olschewski M, Mann JF, Ruf G, Ritz E; German Diabetes and Dialysis Study Investigators: Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. *N Engl J Med* 2005;353:238-248.
- 29 Kawaguchi T, Tong L, Robinson BM, Sen A, Fukuhara S, Kurokawa K, Canaud B, Lameire N, Port FK, Pisoni RL: C-reactive protein and mortality in hemodialysis patients: the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Nephron Clin Pract* 2011;117:c167-c178.
- 30 Tentori F, Blayney MJ, Albert JM, Gillespie BW, Kerr PG, Bommer J, Young EW, Akizawa T, Akiba T, Pisoni RL, Robinson BM, Port FK: Mortality risk for dialysis patients with different levels of serum calcium, phosphorus, and PTH: the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Am J Kidney Dis* 2008;52:519-530.
- 31 Wagner M, Tangri N, Bieber B, Pisoni R, Heuschmann P, Wanner C: Serum albumin and age are the most important predictors of mortality in incident hemodialysis patients – an analysis of the DOPPS cohort. Abstract presented at 17th Congress on nutrition and metabolism in renal disease Würzburg 2014.